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14. ABSTRACT

The goal of this research is to investigate the potential of resveratrol, genistein and (-)-epigallocatechin-3-gallate (EGCG), alone and in combination, to protect against prostate cancer in a transgenic rat model (TRAMP). The specific aims are 1) to investigate the potential of genistein, EGCG and resveratrol, alone and in combination, to suppress the development of spontaneously developing prostate tumors and 2) to investigate the potential of these polyphenols to regulate sex steroid- and specific growth factor- receptor and ligand expression as mechanism of prostate cancer prevention. To date, we have demonstrated that pure resveratrol in the diet, but not EGCG in the water, suppressed spontaneously developing prostate tumors in TRAMPs. Androgen and estrogen receptors and EGF, IGF-1, and ERK signaling pathways are differentially regulated in both the DLP and VP of genistein, resveratrol and EGCG treated mice. We are in the process of investigating combinational genistein and resveratrol treatments to suppress prostate cancer in TRAMPs and to investigate mechanisms of action in mice treated with these 2 polyphenols.

15. SUBJECT TERMS
Genistein, Resveratrol, EGCG, Prostate Cancer Chemoprevention, Mice

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Introduction

Asians consuming a diet high in soy products have reduced incidence of clinically manifested prostate cancers. Likewise, Asians have a long history of drinking tea. Significant components of these two staples of the traditional Asian diet are the polyphenolic compounds. The primary polyphenols associated with prostate chemoprevention are the soy isoflavone, genistein, and the tea catechin, (-)-epigallocatechin-3-gallate (EGCG). Another polyphenol that has recently received attention as a cancer suppressor is resveratrol, a component of grapes. The goal of this research is to investigate the potential of these 3 pure polyphenols, alone and in combination, to protect against prostate cancer. In this manner, it may be possible to ingest moderate amount of each of these foods/chemicals, as opposed to mega amounts of one, and receive an additive or synergistic protective effect without adverse effects with possible elevated exposure.

Body

Aim 1) To investigate the potential of the polyphenols, genistein, EGCG and resveratrol, alone and in combination, to protect against prostate cancer. This is being evaluated in the TRAnsgenic Mouse Prostate adenocarcinoma (TRAMP) model that spontaneously develops prostate cancer.

As previously reported we have demonstrated that genistein suppresses spontaneously developing prostate tumors in rats (1). Recently we found that resveratrol, but not EGCG, was also able to suppress spontaneously developing prostate cancer in TRAMP mice (2005 Report). Based on these results and our originally proposed Task 1 where we stated that we would further investigate in combination those nutritional agents that alone demonstrate efficacy, we are presently investigating the potential of resveratrol and genistein in combination, and not EGCG, to suppress spontaneously developing prostate tumors. Accordingly, the following groups of animals (30/group) have been bred and treated (Task 1, Phase II):

Group 1) Controls fed AIN-76A diet
Group 2) 250 mg Genistein/kg AIN-76A diet
Group 3) 625 mg Resveratrol/kg AIN-76A diet
Group 4) 250 mg Genistein + 625 mg Resveratrol/kg AIN-76A diet

Necropsy of animals was completed in July 2006. The tissues have been processed, sectioned and H&E stained. We await the pathology evaluation and report, hopefully by end of October 2006. The reasons for delay are that our section of Animal Resources facilities was undergoing renovation and there was a subsequent infection in our animal colony. These obstacles have been overcome and now we have generated the animals necessary for the chemoprevention studies.

Aim 2) To investigate the potential of genistein, EGCG and resveratrol to regulate sex steroid- and specific growth factor- receptor and ligand expression as mechanisms of prostate cancer prevention. From the prostates of mice exposed ± polyphenols we will investigate expression of the androgen receptor (AR), estrogen receptors (ERs), epidermal growth factor (EGF) signaling, insulin-like growth factor-I (IGF-I) signaling, and extracellular signaling regulating kinases-1 and 2 (ERK-1 and ERK-2).

Mechanism of action studies with genistein have been completed and published (2).

Resveratrol. In the end of year 2 report (2004-2005) we provided data from the dorsolateral prostate (DLP) tissue only since we did not yet have the ventral prostate (VP) data analyzed. Also since our March 2006 Report, we have carried out more assays.

Resveratrol decreased cell proliferation and reduced the ratio of cell proliferation to apoptosis in prostates of TRAMP mice (Figure 1A). The prostate was evaluated as individual lobes (DLP and VP), as well as in combined prostate lobes. Using the Ki67 assay, we found that resveratrol significantly decreased cell proliferation in the DLP by 50%, in the VP by 31%, and in the entire prostate (DLP + VP combined) by 43% (Fig. 1*A*). Apoptosis assayed by counting apoptotic bodies did not reveal significant differences between the control- and resveratrol-treated animals in the DLP, VP, or entire prostate. When apoptotic indices for resveratrol-treated animals (1.12 ± 0.33) were compared to control apoptotic indices (0.52 ± 0.48), the change in apoptosis in the VP approached statistical significance ($P = 0.053$; Fig. 1*B*). Importantly, however, the cell proliferation to apoptosis ratio was significantly lower in the entire prostate and DLP of resveratrol-supplemented mice. Resveratrol in the diet reduced the ratio of cell proliferation to apoptosis by 72% (3.6-fold), by 69% (3.2-fold), and by 56% (2.3-fold) in the entire prostate, DLP, and VP, respectively (Fig. 1*C*).

Resveratrol did not alter sex steroid concentrations in the blood serum. We quantified sex steroid concentrations in the blood serum of 12-week-old TRAMP mice fed control or resveratrol diet. Total testosterone, free testosterone, estradiol, dihydrotesterone, and sex hormone binding globulin did not differ significantly between resveratrol- and control-treated mice (data not shown).

The sex steroid receptors, AR and ER-beta, but not ER-alpha, were regulated by dietary resveratrol. In the DLP, resveratrol in the diet caused a 2.6-fold increase in AR and a 65% increase in ER-beta protein expressions, but no significant change in ER-alpha (Fig. 2). In the VP of resveratrol treated TRAMP mice, AR and ER-alpha protein levels were not significantly different from those of controls. Because of limited sample volume, ELISA for ER-beta in the VP was not possible.

The IGF-1 signaling proteins were differentially regulated by resveratrol in the DLP and VP. Resveratrol treatment significantly reduced IGF-1 in the DLP, but not in the VP (Fig. 3). On the other hand, resveratrol resulted in up-regulated IGF-1R in the DLP, but down-regulated IGF-1R in the VP. The expression of IGF-BP3, the most abundant binding protein, was not significantly changed in the DLP or VP (data not shown). In addition, we investigated the effect of resveratrol on specific protein expressions in the liver, the major site of IGF-1 production. IGF-1, IGF-1R, and IGF-BP3 protein expression did not differ in the liver between control and treated animals (data not shown).

Resveratrol decreased the expression of ERKs 1 and 2. The protein kinases, ERK-1 (p44 MAPK) and ERK-2 (p42 MAPK) belonging to an extensively studied group of mitogen-activated protein kinases (MAPKs) were measured. Total-ERKs 1 and 2 (phosphorylated and unphosphorylated) remained unchanged in DLP and VP (data not shown); while phospho-ERK 1 (phosphorylated form) was decreased 51% in the DLP and phospho-ERKs 1 and 2 in the VP were down-regulated 34% and 43%, respectively (Fig. 4).

EGCG. We have just completed the analysis of this data.

EGCG down regulated cell proliferation (Ki67) in the VP, but not in the DLP of 12 week old mice (Figure 5A). When the data for the DLP and VP were combined, the total prostate had significantly reduced cell proliferation.

On the other hand, apoptotic indices (apoptotic bodies) were increased in the VP, but not in the DLP (5B). Apoptotic index for the total prostate was also significantly increased.

The ratio of cell proliferation to apoptosis was significantly decreased in the VP and total prostate, but not in the DLP (Figure 5C).

In the DLP, EGCG significantly down-regulated IGF-1, but not AR, ER-α, IGF-1R, phospho-ERKs 1& 2 (Figures 6-10). EGCG treatment down regulated AR, IGF-1, IGF-1R and phospho-ERKs 1 & 2, but did not significantly alter ER-α in the VP. It is interesting that EGCG regulates more sex steroid receptor and growth factor signaling proteins in the VP and yet does not suppress prostate cancer in these animals.

Ongoing work. To investigate sex steroid and growth factor signaling proteins in TRAMP mice exposed to combinational resveratrol and genistein treatments. The groups (8 samples/group; 3 prostates/sample each) are:
Group 1) controls (AIN-76A diet)
Group 2) 625 mg resveratrol/kg diet + 250 mg genistein/kg diet

Resveratrol and genistein treatment is via the diet, starting at conception. The animals have been produced and treatment initiated. The mice will be killed in October 2006. The analyses via western blot analysis and ELISA are projected to be completed in February 2007.

Statistical analysis of histological specimens used Fisher's exact test to determine significance ($P < 0.05$). Analyses were conducted using Microsoft Office Excel 2003 (Microsoft Corp., Seattle, WA). For the biochemical data, experiments were analyzed using one way analysis of variance (ANOVA), with subsequent multiple comparisons. The p-values associated with the individual comparisons were completed using separate t-tests.

Key Research Accomplishments

- Pure resveratrol, but not EGCG, in the diet suppressed spontaneously developing prostate tumors in TRAMPs.
- Resveratrol decreased cell proliferation and reduced the ratio of cell proliferation to apoptosis in prostates of TRAMP mice.
- AR, ER-beta and IGF-1R were up-regulated while IGF-1 and phospho-ERK-1 were down regulated, while ER-alpha and phospho-ERK-2 were not regulated in the DLP by dietary resveratrol.
- In the VP, resveratrol treatment significantly decreased IGF-1R and ERKs 1 & 2 but did not alter protein levels of AR, ER-alpha and IGF-1.
- EGCG did not alter prostate cancer development in TRAMP mice.
- EGCG down regulated cell proliferation in the VP, but not in the DLP.
- EGCG increased apoptotic indices (apoptotic bodies) in the VP, but not in the DLP.

References

1) Mentor-Marcel, R, Lamartiniere, C.A., Greenberg, N. and Elagavish, A. Genistein in the diet reduces the incidence of prostate tumors in a transgenic mouse (TRAMP). Cancer Research, 61:6777-6782, 2001.

2) Wang, J., Eltoum, I.-E. and Lamartiniere, C.A. Genistein regulates growth factor signaling in transgenic mouse model (TRAMP). Molecular and Cellular Endocrinology 219: 171-180, 2004.

Appendices

Eight Figures

- EGCG significantly down-regulated IGF-1, but not AR, ER-alpha, IGF-1R, phospho-ERKs 1& 2 in the DLP.
- EGCG treatment down regulated AR, IGF-1, IGF-1R and phospho-ERKs 1 & 2, but did not significantly alter ER-alpha in the VP.

Reportable Outcomes

Lamartiniere, C.A. Invited presentation to AICR/WCRF International Research Conference on Food, Nutrition and Cancer. Genistein Chemoprevention of Breast Cancer: Timing and Mechanisms of Action, Washington, DC, July, 2005.

Lamartiniere, C.A. Molecular and Cellular Pathology Seminar: Dietary Polyphenols Protect Against Mammary and Prostate Cancers. University of Alabama at Birmingham Department of Pathology. September, 2005.

Lamartiniere, C.A. University of Gottingen, Germany Seminar: Dietary Polyphenols Protect Against Mammary and Prostate Cancers. February, 2006.

Lamartiniere, C.A. Humboldt University, Berlin, Germany Seminar: Dietary Polyphenols Protect Against Mammary and Prostate Cancers. February, 2006.

Lamartiniere, C.A. Technische Universitat, Dresden, Germany Seminar: Dietary Polyphenols Protect Against Mammary and Prostate Cancers. February, 2006.

Lamartiniere, C.A. Invited Speaker at Conference on Aging Men in Salzburg Austria: Dietary Polyphenols Protect Against Prostate Cancers. February, 2006.

Harper, C. Wang, J., Patel, B.J. and Lamartiniere, C.A.. Epigallocatechin-3-gallate (EGCG) down-regulates the androgen receptor and the IGF pathway in the prostate of TRAMP mice. Proceedings of the American Association for Cancer Research. 2006.

Conclusions

Resveratrol in the diet, but not EGCG in the water, suppressed spontaneously developing tumors in TRAMP mice. Of all the sex steroid and growth factor signaling proteins measured, down-regulation of IGF-1, phospho-ERK-1 and up-regulated ER-beta in the DLP and down-regulated IGF-1R and phospho-ERKs 1 & 2 in the VP by resveratrol is consistent with the accepted dogma for chemoprevention since IGF-1, IGF-1R and ERKs are associated with growth factor signaling and cell proliferation. ER-beta has been hypothesized as a suppressor gene/protein. Likewise, decreased cell proliferation favors a protective effect against prostate cancer.

While our long-term tumorigenesis studies do not demonstrate EGCG suppressing prostate tumor development, short term studies demonstrate that EGCG suppresses cell proliferation and enhances apoptosis in the prostate. Furthermore, EGCG significantly down-regulated IGF-1 in the DLP and down-regulated AR, IGF-1, IGF-1R and phospho-ERKs 1 & 2 in the VP. Down-regulation of these sex steroid and growth factor signaling pathways are associated with cancer suppression.

Figure 1A

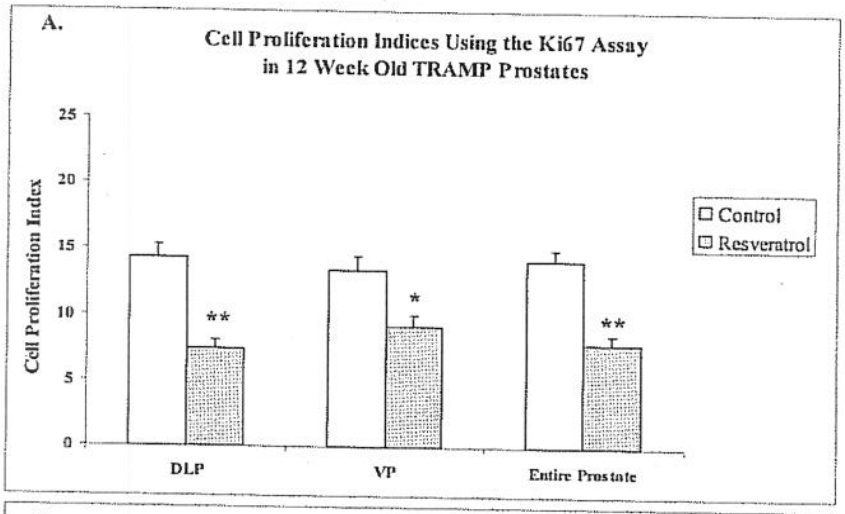


Figure 1B

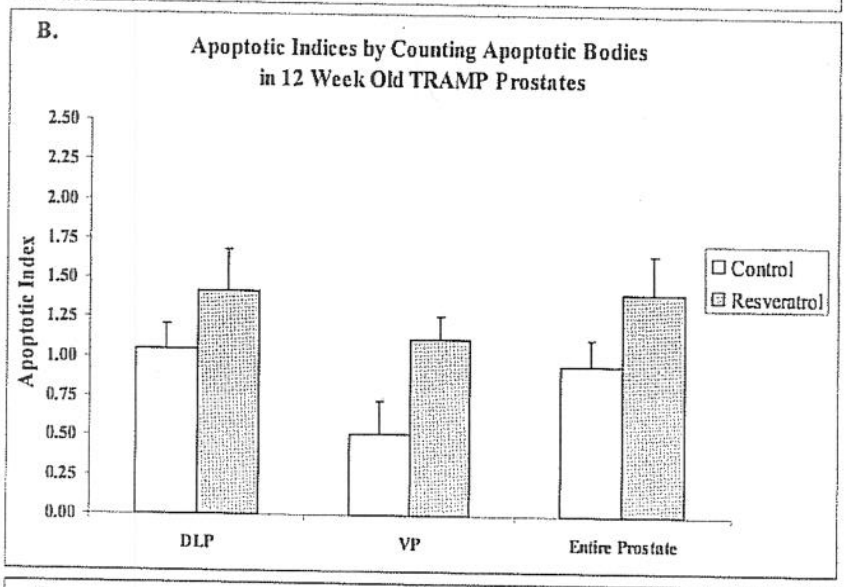


Figure 1C

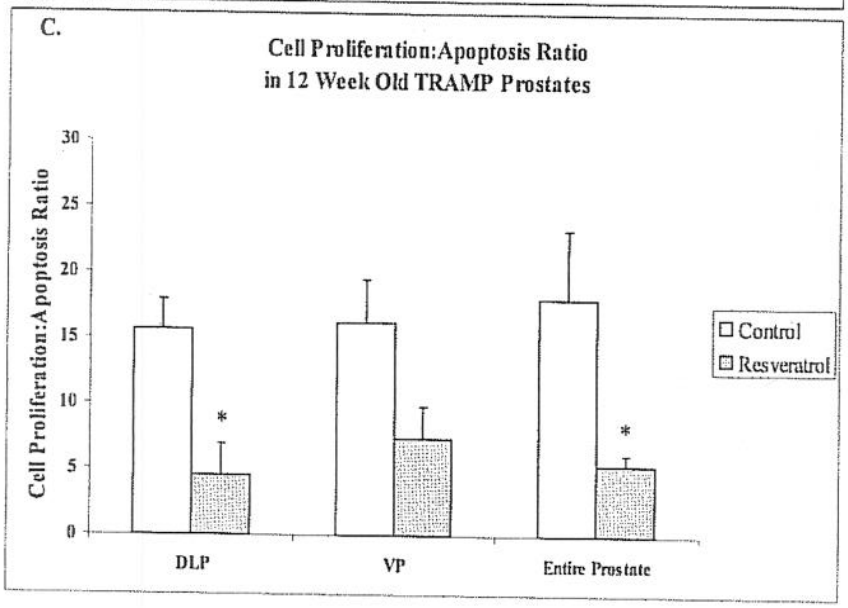


Figure 1. Cell proliferation and apoptosis in the DLP, VP, and entire prostates of 12- week-old TRAMP mice fed AIN-76A diet (Controls) and 625 mg resveratrol/kg AIN-76A diet starting at 5 weeks of age. *A*, data represent the cell proliferative indices. *B*, apoptotic indices. *C*, cell proliferation to apoptosis ratios. $*P < 0.05$, $**P < 0.01$ compared to control treatment.

Figure 2

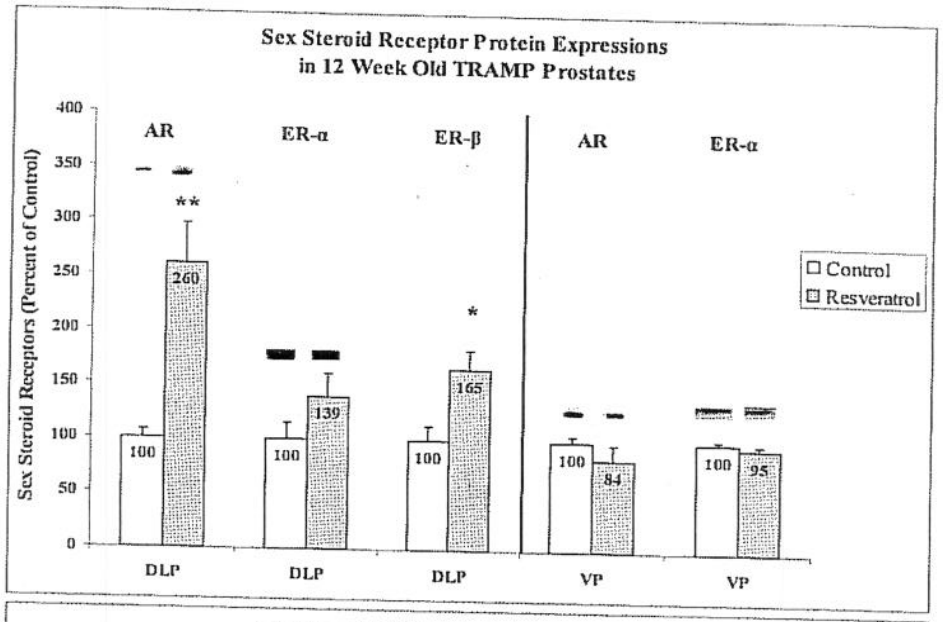


Figure 3

Figure 3

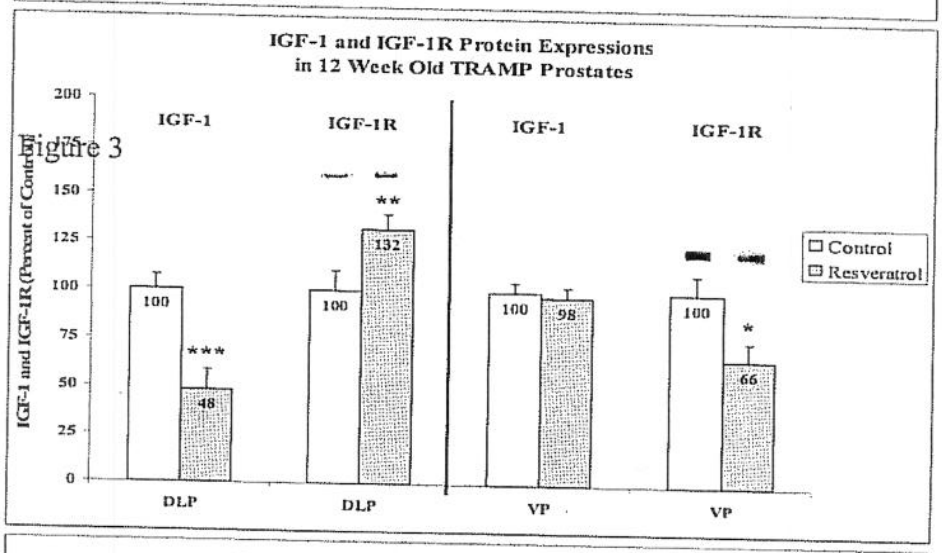
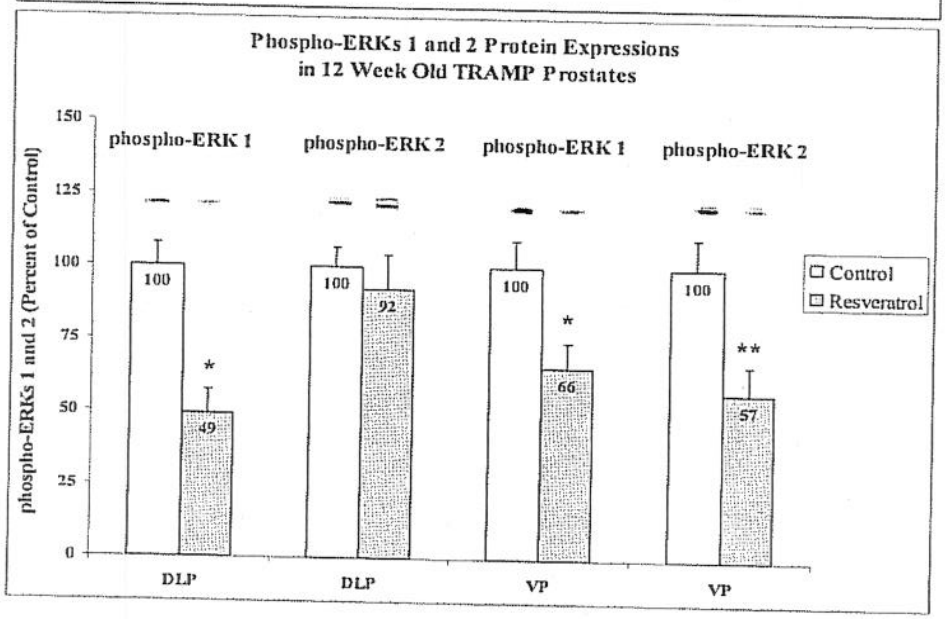


Figure 4



Figures 2-4. Sex steroid and growth factor signaling protein expressions in DLP and VP of 12-week-old TRAMP mice fed AIN-76A diet (Controls) or 625 mg resveratrol/kg AIN-76A diet starting at 5 weeks of age. Upper figures depict representative Western blots. IGF-1 protein expression was determined *via* ELISA. The bar graphs represent data mean ± SEM with control values being set to 100. $^{**}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.01$ compared to control treatment.

Figure 5A

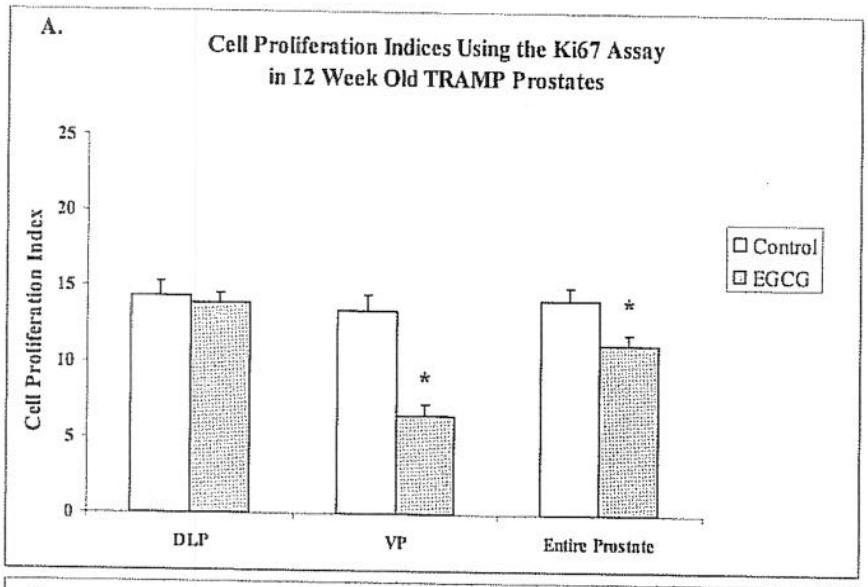


Figure 5B

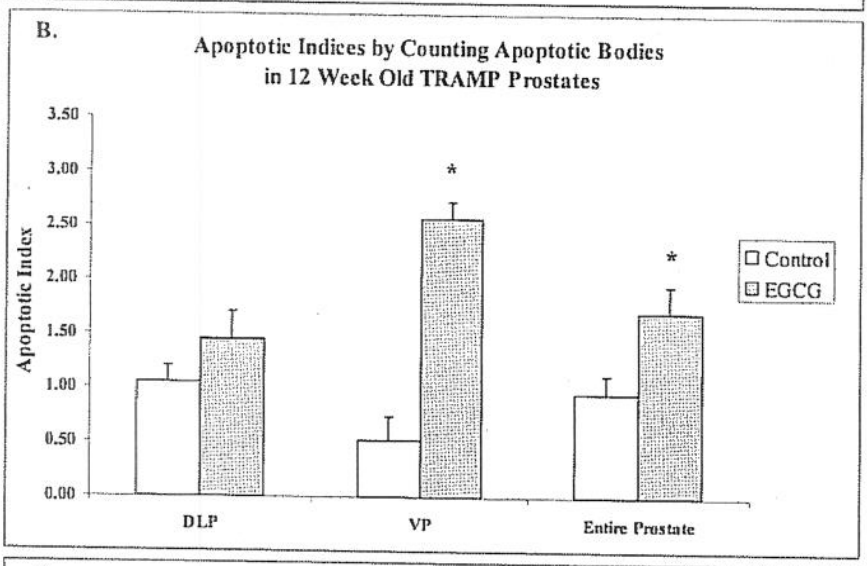


Figure 5C

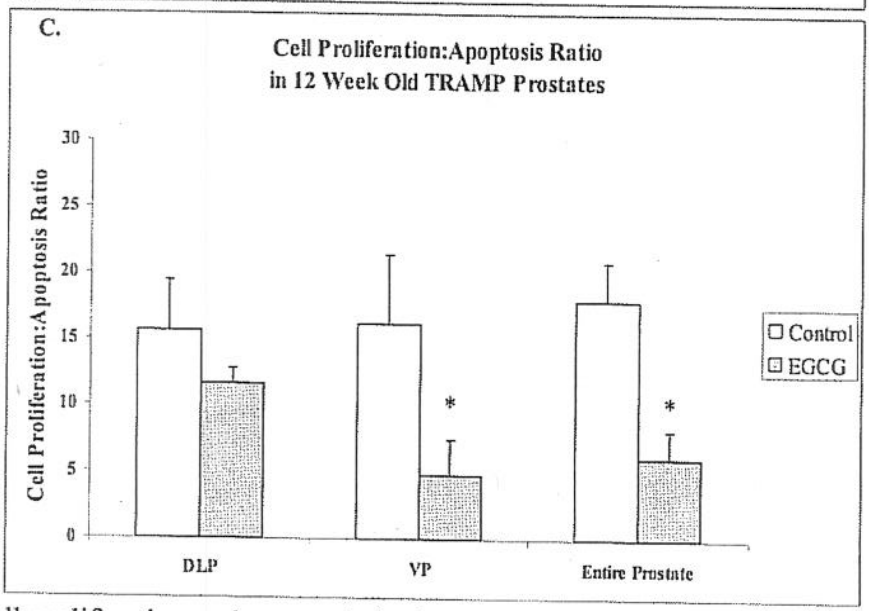


Figure 5. Cell proliferation and apoptosis in the DLP, VP, and entire prostates of 12- week-old TRAMP mice fed AIN-76A diet and tap water (control) or 0.06% EGCG in tap water starting at 5 weeks of age. *A*, data represent the cell proliferative indices. *B*, apoptotic indices. *C*, cell proliferation to apoptosis ratios. $*P < 0.05$ compared to control treatment.

Figure 6

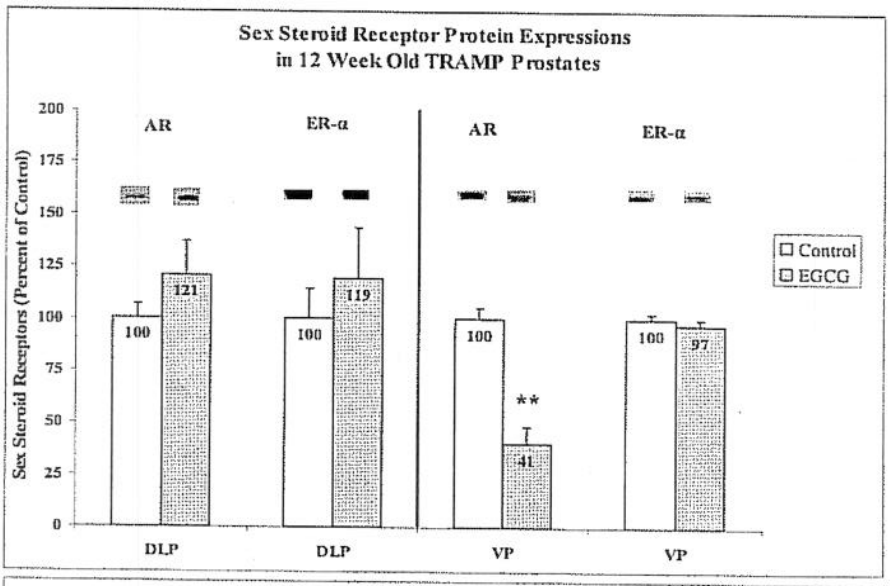


Figure 7

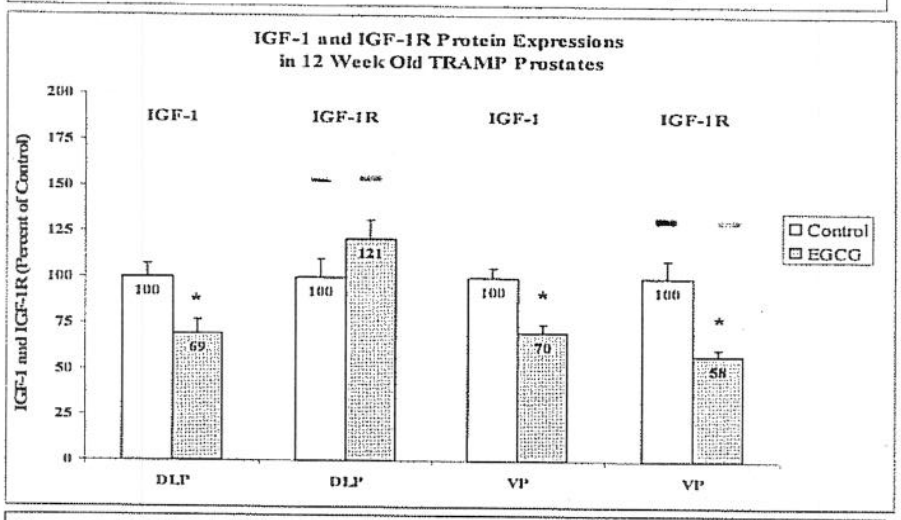
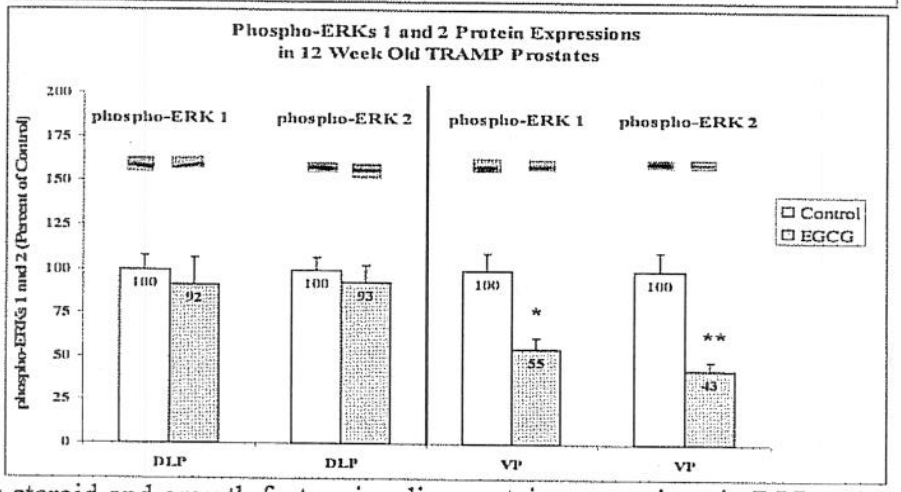


Figure 8



Figures 6-8. Sex steroid and growth factor signaling protein expressions in DLP and VP of 12-week-old TRAMP mice fed AIN-76A diet and tap water (control) or 0.06% EGCG in tap water starting at 5 weeks of age. Upper figures depict representative Western blots. IGF-1 protein expression was determined *via* ELISA. The bar graphs represent data mean ± SEM with control values being set to 100. $^{**}P < 0.05$, $^{**}P < 0.01$ compared to control treatment.